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The prevalence of Trichinella spiralis in farmed minks (Neovison vison) associated with exposure to wild rats (Rattus norvegicus) in Shandong province, China

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Abstract

Background Both of American mink (*Neovison vison*) and wild rat (*Rattus norvegicus*) is considered reservoir hosts carrying many endoparasites. *Trichinella* is a successful parasitic nematode including nine species and three genotypes with a worldwide distribution. However, little is known about the prevalence of *Trichinella* infection in mink (*Neovison vison*) and rat (*Rattus norvegicus*) in China.

Methods In total, 289 muscle samples of minks and 102 rat carcasses were collected between April 2017 and December 2019 in Weihai city of Shandong province, China. The food of minks including chicken skeleton and mashed marine products was also collected at 3 batches. All the samples were used to examine for the appearance of *Trichinella* by the pooled artificial HCl-pepsin digestion method. The isolates from minks and rats were identified as *T. spiralis* by multiplex PCR. Then, the phylogenetic tree was constructed based on the sequences of 5S rDNA inter-gene spacer regions.

Results Muscle larvae were detected in 20 out of 289 minks (6.92%) and in 2 of 102 wild rats (1.96%), respectively. The intensity of *Trichinella* in mink samples was ranged from 0.025 to 0.815 lpg, while the larval burden in rats was 0.17 lpg. The isolates from minks and rats were identified as *T. spiralis* by multiplex PCR. Sequence analysis revealed a 100% identical alignment of the 5S rDNA inter-gene spacer regions from the two isolates. The phylogenetic tree confirmed the two isolates from minks and rats belonging to *T. spiralis* based on analysis of the 5S rDNA inter-gene spacer sequence.

Conclusions The present study represents the first report of *T. spiralis* infection in American mink (*Neovison vison*) and wild rat (*Rattus norvegicus*) from Shandong province, China. The farmed minks would be vulnerable to *Trichinella* infection through exposure to the wild rats. The prevalence of *T. spiralis* in wild rats may raise a public health concern for the potential zoonotic risk for the domestic animals.

Background

The zoonotic trichinellosis is a parasitic disease of public health significance, caused by infection with larvae of the genus *Trichinella* [1]. The parasite can infect more than 150 animal species including birds, mammals and reptiles [2, 3]. To data, there are nine species and three genotypes consisting of *Trichinella* genus [4]. In China, trichinellosis is still recognized as a re-emerging disease [5], and more than 600 outbreaks were reported during 1964–2013, affecting nearly 40 thousand people [6]. The high prevalent setting of human trichinellosis in China is mainly related to the consumption of raw or under-cooked pork, and sporadically to vagarious ingestion of meats from dogs and wild animals as delicacies [7].

American mink (*Neovison vison*) is one of important economic animals for providing better marten, which is very popular in international fur market. Breed of minks is more increasing in China due to the abundant economic benefit [8]. Owning the largest breeding industry of minks in China, more than 70 percent of marten are yielded in Shandong province, especially around the coastal regions including Yantai, Weihai and Qingdao cities. Meanwhile, mink is also considered a crucial reservoir host for endoparasites in view of its wide food niche breadth (fish, birds, amphibians, small mammals and

invertebrates) [9, 10]. Several investigations have showed *Trichinella* infection in the farmed minks in other countries, but data are limited on the prevalence of *Trichinella* in farmed minks (*Neovison vison*) in China.

In China, free-ranging pigs are at high risk for *Trichinella* spp. infections in humans in the place without any mandatory test [11, 12]. Meanwhile, the domestic animals are assailable to *Trichinella* infection through exposure to wild reservoir hosts. Wild rats (*Rattus norvegicus*) can act as the primary reservoir host of *Trichinella* that trigger the onset of the transmission of zoonotic bacteria, viruses or parasites to the domestic cycle derived from the sylvatic environment when the carcasses are scavenged [13–15]. Rats commonly abundant lives wherever humans live, especially in primitive conditions less than desirable hygiene. However, little report could be available about *Trichinella* prevalence in wild rats (*Rattus norvegicus*) in China.

In this survey, we aimed to examine the prevalence of *Trichinella* infection in farmed American mink (*Neovison vison*) populations from Weihai city of Shandong province, China, and determined species of the isolates by multiplex PCR and phylogenetic analysis. In order to further investigate the source of infection, we examined *Trichinella* by artificial digestion of the diets of minks including mixed mash and chicken skeleton, and the rats living closely with minks.

Methods

Sample collection

The investigation of *Trichinella* infection from 289 minks (*Neovison vison*) was conducted between April 2017 and December 2019 in Weihai city (36°41' ~ 37°35' N, 121°11' ~ 122°42' E) of Shandong province (Fig. 1). Each mink was raised in a cage, farming at a semi-closed house. Samples used for testing mink (*Neovison vison*) were included tongue, diaphragm, forearm and hind limbs, and muscle tissues of 5 ~ 50 g were taken from one animal [16]. A batch of muscle tissues was stored at 4 °C, keeping no more than 15 days. Due to the minks were fed on chicken skeleton and mixed mash (mainly including little sea-fish, shrimp et al.), we collected 600 g of mixed mash and 800 g of chicken skeleton by 3 batches.

Rats (*Rattus norvegicus*) (*n* = 102) around/inside mink farm were trapped between January and December 2019 using mousetraps that placed surrounding the rat holes. The captured rats were kept frozen at -20 °C until examination. The carcass of rats was obtained before laboratory analysis.

If needed, male inbred Kunming mice (6–8 weeks) were purchased from Center of Laboratory Animals, Lanzhou Veterinary Research Institute, Lanzhou, China. All mice were handled in strict accordance with the Good Animal Practice requirements of the Animal Ethics Procedures and Guidelines of the People’s Republic of China.

Parasitological test by artificial digestion

Samples from minks (*Neovison vison*), mixed marine mash, chicken skeleton or rat carcasses were used to examine for the present of *Trichinella* by the artificial HCl-pepsin digestion method, following as Gamble et al. (2000) [17]. For preparing sample by blending to digestion, up to 100 g of mixed mash, chicken skeleton, or rat carcass, and 200 g of muscle tissues from minks is minced, and mixed with an equal volume of artificial digestion fluid [1% pepsin (1: 10000, Sigma, USA), 1% HCl and 0.9% NaCl]. The digestion of samples was used the magnetic stirring method, maintaining at 45 °C for 2 h.

The storage and maintenance of *Trichinella* isolates

Obtained muscle larvae were washed five times in 0.9% NaCl, and then were examined the morphological characteristics under light microscope with 40 × magnification. The loadings of *Trichinella* were calculated based on the larvae per gram (lpg).

The isolated larvae were stored in 70% ethanol for further species identification by multiplex PCR if the worm is limited. The isolated larvae were recovered through oral infection of Kunming mice using at least 50 larvae for each mouse, and the muscle larvae were obtained by digestion from Kunming mice if needed.

T. spiralis Henan isolate, *T. pseudospiralis* Russin and *T. pseudospiralis* strain (ISS141, T4) was maintained in Kunming mice as described by Fu et al. (2009) [18].

Preparation of genomic DNA and PCR assays

Genomic DNA was extracted with the TIANamp Genomic DNA Kit (TIANGEN, China) according to the manufacturer's instruction. The *Trichinella* species were identified by multiplex PCR [19], using primers of 5'-GTTCCATGTGAACAGCAGT-3' and 5'-CGAAAACATACGACAACTGC-3'. The PCR amplification was performed using the 2 × Unique™ Taq MasterMix (Novogene, China) according to the manufacturer recommendations, consisting of DNA 1 µL, 2 × Unique™ Taq MasterMix 12.5 µL, 10 µM of each primer, and H_2O up to 25 µL. The PCR cycle condition was performed as following: pre-degeneration at 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 58 °C for 30 s, and 72 °C for 1 min, and 1 extension cycle at 72 °C for 4 min. The PCR products were separated on 1% (w/v) agarose gels, stained with GoldenView™.

Sequencing and phylogenetic analysis of the 5S rDNA inter-gene spacer region

For phylogenetic analysis, the 5S rDNA inter-gene spacer region of *Trichinella* was amplified according to our previous description [18], using primers as following: PF: 5'- TTGGATCGGAGACGGCCTG – 3' and PR: 5'- CGAGATGTCGTGCTTTCAACG – 3'. All the primers were synthesized by Invitrogen Company, China. The PCR reaction system was performed in a volume of 25 µL, consisting of DNA 2 µL, 2 × Unique™ Taq MasterMix 12.5 µL (Novogene, China), 10 µM of each primer, and H2O up to 25 µL. The thermal cycling condition was performed as following: pre-amplification cycle at 94.8 °C for 5 min, followed by 35 cycles of 94.8 °C for 1 min, 55.8 °C for 1 min, 72.8 °C for 1 min, and followed by the last cycle at 72.8 °C for 10 min.

The PCR products derived from 5S rDNA inter-gene spacer region were directly sequenced by the Shanghai Songon Biological Engineering Biotechnology Company, China. The obtained sequences were aligned with the corresponding sequences from *T. spiralis* Henan isolate, *T. pseudospiralis* Russin and *T. pseudospiralis* strain (ISS141, T4) amplified in this study, as well as from *T. spiralis* ISS623 (accession no.: GU339129), *T. spiralis* ISS154 (accession no.: GU339131.1), *T. spiralis* ISS31 (accession no.: GU386314.1), *T. spiralis* ISS1222 (accession no.: GU339135.1), *T. spiralis* ISS03 (accession no.: KM357422), *T. nelsoni* ISS37 (accession no.: KM357416.1), *T. patagoniensis* ISS2311 (accession no.: MF668227.1), *T. nativa* (accession no.: AP017702.1), *T. britovi* ISS120 (accession no.: KM357413.1), *T. murrelli* ISS417 (accession no.: KM357414.1), *T. papuae* ISS1980 (accession no.: KM357417.1), *T. pseudospiralis* ISS176 (accession no.: KM357410.1), *Trichinella* sp. T6 (accession no.: JQ511989.1), *Trichinella* sp. T8 (accession no.: EF517128.1) and *Trichinella* sp. T9 (accession no.: KM357420.1) getting from GenBank. The alignment was performed by ClustalX 1.83 [20]. Phylogenetic tree was reconstructed using maximum parsimony (MP) method based on the sequences derived from 5S rDNA intergene spacer region of different *Trichinella* isolates. The MP method was performed using MEGA 5.05.

Results

Isolates from minks (*Neovison vison*), mixed mash, chicken skeleton and rats (*Rattus norvegicus*)

After artificial digestion, *Trichinella* were obtained from minks and rats, but were not detected in mixed marine mash and chicken skeleton. The overall rates of *Trichinella* infection in minks (*Neovison vison*) and rats (*Rattus norvegicus*) were 6.92% (20/289) and 1.96% (2/102), respectively. Thereinto, about 150 larvae were isolated from a blending muscle tissue of minks, and then were reserved in Kunming mice by oral. The lpg of infected mink samples was ranged from 0.025 to 0.815. The larval burden in collected rats was 0.17 lpg.

Identification of *Trichinella* species by multiplex PCR

The obtained larvae were identified species by multiplex PCR. As shown in Fig. 2, the size of PCR production of larvae from mink and rat samples was identical to that of *T. spiralis* Henan isolate with approximate 200 bp in length, which was coincided with the expected size (173 bp) for *T. spiralis* as reported. The fragments from *T. pseudospiralis* Russin and *T. pseudospiralis* ISS141 were longer than 300 bp, respectively. The result indicated that the larvae isolated from American minks (*Neovison vison*) and wild rats (*Rattus norvegicus*) should be identified as *T. spiralis.*

Sequencing and phylogenetic analysis of 5S rDNA inter-gene spacer region

The sequencing result of 5S rDNA inter-gene spacer region from minks was showed 100% identical with that from rats. The length of amplified 5S rDNA inter-gene spacer region from minks (accession no. in GenBank: MT734668) and rats (accession no. in GenBank: MT734669) was 568 bp after fragment

assembly. The 5S rDNA inter-gene spacer region from minks revealed 1 transition (522 T to C) in alignment with the corresponding sequence from Henan isolate.

Phylogenetic analysis using MP based on the 5S rDNA inter-gene spacer region showed that *Trichinella* isolates from minks and rats were all grouped with *T. spiralis* strains, which further indicated they were belonged to *T. spiralis* (Fig. 3).

Discussion

The prevalence of *Trichinella* in minks has been reported in many countries [21–23]. In the present study, we reported the first data on *Trichinella* infection in farmed American minks (*Neovison vison*) in China. The overall *Trichinella* prevalence in minks here was 6.92%, which was lower than that in minks from Estonia (23%) [9] and Canada (8.3%) [24], but higher than that in minks from Poland (3.3%) [10] and Belarus (4%) [25]. The difference in *Trichinella* prevalence in minks among studies would be resulted from the simple size, geographic region, living environment, climatic conditions, as well as animal husbandry practices, socioeconomic and ecological conditions.

The species of *Trichinella* isolates from minks and rats were all identified as *T. spiralis* in the present study, which was different from another previous investigation, isolated *T. britovi* and *T. pseudospiralis* two more strains in minks [10]. *T. spiralis* is considered a preponderant species in China, and as many as 13 isolates are identified as *T. spiralis* obtained from mainland China until now [7]. After isolated *T. spiralis* from caged minks, we raise a question as to how the minks acquired *Trichinella*, the unique parasite that transmit to other animals solely by ingestion of muscles infected with encysted larvae [15]. The food of minks was then conducted to examine the presence of *Trichinella*, but no worms were detected.

Each investigated mink raises in one cage. The cages are setting under a semi-closed house, which one side of the house orientate to the open, facilitating the keepers add mink diets to the food box. In the mink farm, there are many rat holes around the feeding house. According to the keepers, rats often grab the diets of minks from the food box. The fact that the stealing rats could be suddenly preyed by minks sometimes provides the other possible route of minks acquired *Trichinella*. After artificial digestion, *T. spiralis* was obtained from rat carcass, and was identified 100% identity of the partial 5S rDNA inter-gene spacer region to the mink isolate. We thus deduced that, for minks, *T. spiralis* could be transmitted from wild rats, although it should not be the only route.

Minks have been showed to carry Hepatitis E virus [26], SARS-CoV-2 [27], *Cryptosporidium* spp. [28] and several other zoonotic pathogens [29–32]. In the present study, we identified the presence of foodborne *Trichinella* in farmed caged minks. Even though the zoonotic *T. spiralis* infection does not influence the furry quality, the carcasses of minks would increase the biomass of *T. spiralis* in the surrounding environment, if they are not adequately destroyed.

The overall prevalence of *Trichinella* infection in rats from Weihai city of Shandong province was 1.96%, which is lower than that in rats from Northwest Vietnam (2.8%) [33] and Poland (23.33%) [34]. *T. spiralis* infection in wild rats is also well investigated in Europe [35, 36] and America [37]. However, the prevalence of *Trichinella* species circulating is usually overlooked in rats in China. The rats could extensively live from the sylvatic to the domestic environment, so as to play as a vector of *Trichinella* transferred the parasite between domestic animals and wildlife [15, 35]. Although the low larval intensity is detected in the investigated rats (0.17 lpg), presence of *T. spiralis* in rats indicated that attention needs to raise for the pig and chicken farms in view of the public health concerns.

Conclusions

The present study firstly demonstrated *T. spiralis* prevalence in minks and rats from Weihai city of Shandong province, China. The wild rats would be responsible for the caged minks acquired *T. spiralis* in the ecological and genetic viewpoint. Supervisions and inspections of destroying mink carcasses should be urgent for veterinarians, farmers and governmental authorities, and also the prevalence of *T. spiralis* in rats should be further assessed its risk for the domestic animals.

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

N-ZZ conceived the study. NZZ, BQF, WHL, HJY designed the study protocol. NZZ, HJY, DYN, WGC, YJL, HTQ, LL performed the experiments. NZZ, WZJ, HBY analyzed the data. NZZ drafted the manuscript. BQF and WHL contributed to revise the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The 5S rDNA inter-gene spacer sequences of *Tichinella spiralis* from American minks and wild rats have been deposited in the GenBank under the accession numbers MT734668 and MT734669.

Ethics approval and consent to participate

All procedures involving animals in the present study were approved and this study was approved by the Ethics Committee of the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural

Sciences (Approval No. LVRIAEC2019-028).

Consent for publication

Not applicable.

Competing interests

All authors declare no potential conflicts of interest and no sources of support.

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Figures

Figure 1

Map of China with the studied locality. The red area represents the location of the investigated location, Weihai city of Shandong province. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

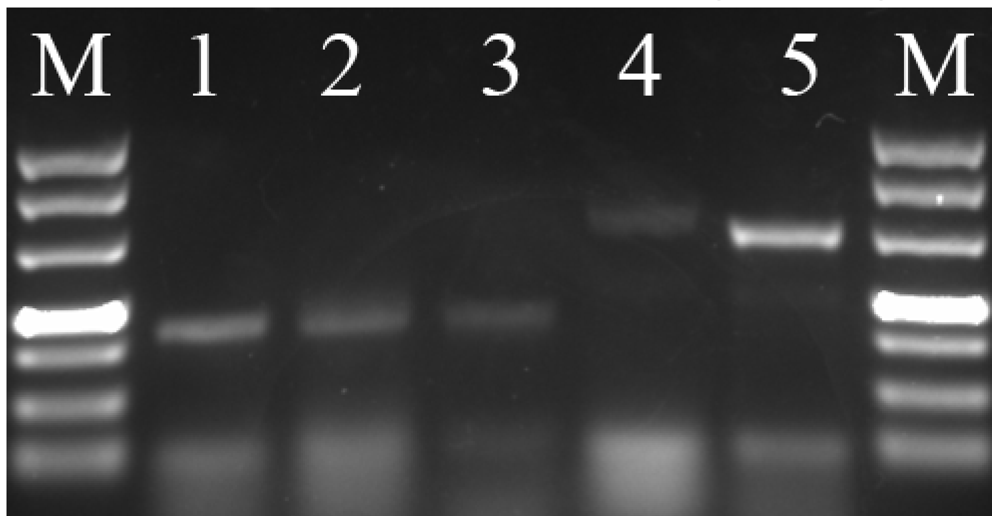

Figure 2

The multiplex PCR amplification of muscle larvae of Trichinella. M: DNA marker (DL500); line 1: T. spiralis Henan strain; line 2: Isolate from American mink (Neovison vison) in the present study; line 3: Isolate from wild rat (Rattus norvegicus) in the present study; line 4: T. pseudospiralis strain (ISS141, T4); line 5: T. pseudospiralis Russin

Figure 3

Phylogenetic analysis of Trichinella spp. based on 5S rDNA inter-gene spacer regions. The tree was built by maximum parsimony (MP) analysis.